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Effect of acupuncture on inflammatory cytokines expression of spastic cerebral palsy rats

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ABSTRACT

Objective: To To investigate the effect of acupuncture on the tumor necrosis factor-α (TNF-α), interleukin -6 (IL-6), C-reactive protein (CRP), nitric oxide synthase (NOS) content and muscular tension of spasticity cerebral palsy rat model. **Methods:** The rats with spastic cerebral palsy were randomly divided into the control group, model group and acupuncture group. After successful modeling, the muscular tension and the content of TNF-α, IL-6, CRP, NOS were measured. **Results:** The serum TNF-α, IL-6, CRP, NOS content were significantly decreased in the acupuncture group ($P<0.05$). The low and high shear viscosity of whole blood of the acupuncture group were significantly lower than the control group and the model group ($P<0.05$). The erythrocyte electrophoresis indexes in the acupuncture group were significantly lower than that in the model group and the control group ($P<0.05$). Acupuncture significantly reduced the muscular tension of spastic cerebral palsy rat and increased the active extent in the paralytic extremity ($P<0.05$), but it could not be restored to normal level. Compared with the control group, the difference had significant ($P<0.05$). **Conclusions:** Acupuncture treatment can inhibit the release of inflammatory cells after brain injury, then reduce immune injury, relieve muscle spasms and reduce muscular tension.

1. Introduction

Cerebral palsy refers to the non-brain damage caused by various reasons within one month antenatal and postnatal, which has different clinical manifestations according to the damage location. Among them, the spastic cerebral palsy which involves the pyramidal tract system has the highest incidence. The main clinical manifestations are limb spasm and muscular hypertonia. The clinical effect of acupuncture is obvious, which can reduce muscular tension and improve motor function[1]. But there is little research on spastic cerebral palsy. In this study, the spastic cerebral palsy rats were used as experimental subjects. We observed the effect of acupuncture on inflammation and muscular tension, and then further explore the mechanism of acupuncture to improve spastic cerebral palsy.

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2. Materials and methods

2.1 Animals and materials

A total of 60 healthy SPF Wistar male rats were selected, weighing 160-200 g. All animals were provided by the XX provincial medical laboratory animal center, kept in the SPF animal room, with constant temperature 25 ℃ and constant humidity 40%-50%. Tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP), nitric oxide synthase (NOS) ELISA kits were purchased from American R & D companies. WQ9E pain threshold detector was purchased from Shanghai sixth medical equipment plant, WLMS-2A two channels physiological recorder and arterioles clip were purchased from Chengdu Instrument Factory.

2.2. Methods

2.2.1. Grouping and modeling methods

According to random number table, 60 male Wistar rats were randomly divided into the control group, the model group and the acupuncture group (n=20). All died or abandoned animals in midway were supplemented by modeling again. All rats received adaptive breeding for one-week in animal room after back from the Experimental Animal Center. The control group was fed with water and the standard diet. Rats were restrained in a plank for 30 min every day and fixed every 12 h for 14 days. According to Wang *et al*[2], in model group two vessel occlusion method was used to produce spastic cerebral palsy rat model. The animals were anesthetized with intraperitoneal injection 100 g/L chloral hydrate (350 mg/kg). Skin was preserved by sterilizing the anterior neck skin then they were cut. The common carotid arteries in both sides were separated along with both sides of the trachea, then threading and suspension was carried out. After bilateral common carotid arteries clamping for 20 min, blood sample was obtained from mice tail end, and the blood volume was 10% (6 mL/kg). After micro-artery clamp removing and infusion for 10 min the bilateral common carotid arteries were clamped again. It was repeated for three times and wounds were sutured. Then all animals received intraperitoneal injection of sodium penicillin 40 000 U/kg to prevent infection. After anesthesia, rats were restrained in a plank for 30 min every day from the first day of modeling and fixed every 12 h for 14 days. The modeling method of the acupuncture group was the same as above. And acupuncture was sutured from the first day of modeling. Rats were treated by acupuncture on point "Dazhui", "Bai Hui" and "Renzhong". The acupuncture points were in accordance with the method provided by "Experimental Acupuncture" written by Li et al and "Experimental Animal Atlas" by Hua et al. During acupuncture, per point for 1 min, they were twirled, and the needles were retained for 30 min. It was performed every 12 h for 14 days[3].

2.2.2. Model determination

After the animals were awake, neurological symptoms were assessed by the modified Tarlov grading method: Completely paralyzed without any movement is grade 0; Severe paralysis, only slight movement, without crawl or ability to support the body is grade 1; Severe paralysis, without crawl, but with ability to support the body is grade 2; Mild paralysis, with ability to support the body, unsteady gait with limp is grade 3; Walking normally, no paralysis is grade 4[4]. Muscle tension was assessed by using the modified Ashworth Scale Assessment method: Rats with normal activities and normal muscle tension is grade 0; transient pause during the activity process is grade 1; easy limb flexion and extension, but with mild ataxia is grade 2; difficult limb flexion and extension, with moderate ataxia is grade 3; limited limb flexion and extension, with severe ataxia is grade 4[5]. The successful modeling of spastic cerebral palsy rats is grade 3 or less than grade 3 paralysis, with grade 1 or above grade 1 muscle tension[6].

2.2.3. Index detection

The electrical stimulation needle was inserted into the quadriceps femoris, and the other end was connected to the pain threshold detector to measure muscle tension.

A thread was tied to the lower end of hind limb, the other end was tied to the two channels physiological recorder. The quadriceps femoris was stimulated and hindlimb straighten length was recorded. Then heparin disposable 5 mL was used with sterile syringe to obtain 2 mL blood at point of maximal impulse through percutaneous puncture into the EP tube. Serum TNF-α, IL-6, CRP and NOS was measured by radioimmunoassay. The whole blood viscosity, plasma viscosity, erythrocyte electrophoresis index and the erythrocyte aggregation index were determined[7].

2.3. Statistical analysis

Data were expressed as mean±SD values. All data was analyzed with SPSS 13.0 software. After the variance test, the difference between two groups was compared with single factor analysis of variance. P<0.05 was regarded as statistical significance.

3. Results

3.1. Serum TNF-α, IL-6, CRP, NOS levels

TNF-α, IL-6, CRP, NOS were significantly increased in the model group, compared with the in the control group (P<0.05). TNF-α, IL-6 levels of the acupuncture group were significantly higher than that in the control group (P<0.05), while were significantly lower than that in the model group (P<0.05). There was no significant difference in CRP, NOS level between the acupuncture group and the control group (P>0.05), while were significantly lower than that in the model group, (P<0.05) (Table 1).

Table 1
Comparison of rat serum TNF-α, IL-6, CRP, NOS levels.

Items	Control group	Model group	Acupuncture group
TNF-α (ng/mL)	2.1±0.2	2.6±0.4*	2.4±0.2*△
IL-6 (ng/L)	50.4±12.8	122.5±28.9*	66.3±18.5*△
CRP (μg/L)	1.4±0.4	2.3±1.0*	1.2±0.4△
NOS (μ/mL)	23.5±2.1	27.4±2.2*	24.9±1.9△

Note: * Compared with the control group, P <0.05, △ compared with model group, P<0.05.

3.2. Eeffects of acupuncture on blood rheology

There was no significant difference in blood rheology index between the model group and the control group (P>0.05). The whole blood high shear viscosity and low shear viscosity of the acupuncture group was significantly lower than the

control group and model group ($P<0.05$). There was no significant difference in plasma viscosity or erythrocyte aggregation index among acupuncture group, acupuncture group and model group and the control group ($P>0.05$) (Table 2).

3.3. Effect of acupuncture on muscular tension of spastic cerebral palsy rats

The muscular tension of spastic cerebral palsy rat increased significantly, compared with the control group ($P<0.05$). Acupuncture could significantly reduce the muscular tension of spastic cerebral palsy rat and increased the active extent in the paralytic extremity. Compared with the model group, the difference was statistically significant ($P<0.05$), but it could not restore to normal level. Compared with the control group, the difference was significant ($P<0.05$) (Table 3).

4. Discussion

Spastic cerebral palsy belongs to the "five delays", "five weaknesses ", "five stiffness" category in TCM, including retardation, slow movement, limb spasm, stiff hands and feet, muscular hypertonia, abnormal posture and other clinical manifestations. It is located at channels and vessels of the brain. Its causes is the lack of congenital fetal development, maternal weakness or exogenous factors, or the acquried hurt led to blood-stasis stagnated in orifice, meridian obstruction and marrow dystrophy and related to the yin-yang disharmony of both kidney, spleen and liver, eventually leading to obstruction of qi and blood circulation, malnutrition of the skin and arteries and veins, or yin deficiency, meridian cross channeling, retention in the brain, thus forming the excess in upper and deficiency in lower and imbalance of yin and yang[8-10].

Therefore, according to this pathogenesis, it can be treated by consciousness-restoring resuscitation, Fu-dredging and turbidity-purgating, supplementing qi and activating blood circulation, promoting blood circulation to remove collaterals obstruction and other methods. Acupuncture treatment with unique features and good effect has been widely used even till today. With the deep and lasting understanding of the pathophysiology of spastic cerebral palsy, we found that because of the damage of the cerebral cortex and related structures, negative-feedback inhibition at the supraspinal level is basically in lack, leading to the imbalance between the suppression and excitement loop which regulate skeletal muscle shrinkage, then spasticity occurred. Because spastic cerebral palsy occurs one month antenatal and postnatal, in this study we selected 6-7 weeks adult rats, combined the bilateral carotid artery ligation with obtained blood from mice tail end method. Although not fully comply with the pathological changes of spastic cerebral palsy, but the model has the basic characteristics of upper motoneuron injury, basically meet the test requirements of spastic cerebral palsy[11-14].

The study detected the serum inflammatory factors and blood rheology indicators of spastic cerebral palsy rat after acupuncture, and found that acupuncture treatment can improve the brain blood supply disorder, increase blood flow to the ischemic area, reduced inflammatory cytokines levels of tissue and reduced the inflammation, also with activated brain tissue self-protection mechanism, then inhibited ischemia, inflammation or a serial cascade of pathophysiologic reaction etc. Previous studies have shown that spastic cerebral palsy is because of the lack of inhibition of upper motor neuron to lower motor neurons, which can cause muscular tension imbalance[15-17]. The main neurotransmitter to maintain muscular tension is the GABA, and the brain damage significantly reduced GABA release. Studies suggest that acupuncture "Baihui" point can inhibit penumbra nerve cells, which can significantly inhibit neuronal apoptosis of penumbra. Acupuncture "Dazhui", "Baihui", "Renzhong" can inhibit the excessive excitement of thesympathetic nerve, reduce the ischemic neuronal excitability of insular cortex, which can significantly

Table 2

Comparison of rheology before treatment in each group (mPa•s).

Groups	Whole blood high shear viscosity	Whole blood low shear viscosity	Plasma viscosity	Erythrocyte aggregation index	Erythrocyte electrophoresis index
Control group	5.5±0.5	16.7±1.9	1.4±0.1	2.9±0.1	5.8±0.3
Model group	5.6±0.4	17.2±1.8	1.5±0.2	3.1±0.2	6.2±0.3
Acupuncture group	5.2±0.5*△	15.4±1.3*△	1.4±0.1	3.0±0.1	5.9±0.3△

Note: * Compared with the control group, $P<0.05$, △ compared with model group, $P<0.05$.

Table 3

Muscular tension of spastic cerebral palsy rats.

	Groups	n	Muscular tension (grade)	Active extent in the paralytic extremity(mm)
Control group	Before treatment	20	0	21.5±2.2
	After treatment	20	0	21.4±2.1
Model group	Before treatment	20	2.8±0.9	12.5±2.8
	After treatment	20	2.7±0.9	13.2±2.9*
Acupuncture group	Before treatment	20	2.9±0.8	12.4±2.9
	After treatment	20	1.1±0.4	18.1±2.7*△

Note: * Compared with the control group, $P<0.05$, △ compared with model group, $P<0.05$.

improve symptoms of cerebral ischemia reperfusion[8-20].

During the cerebral ischemic process, the overexpression of some inflammatory cytokines such as TNF- α , IL-6, CRP, NOS can activate endothelial cells to enhance inflammation and promote leukocyte infiltration, lead to blockage of capillaries, leakage and reduce capillary blood flow, which can cause blood stagnation of the ischemic area, infiltrating leukocytes to release oxygen free radicals, soluble protein and cytokinin then leading to cell necrosis. Acupuncture treatment can reduce the inflammatory cytokines levels and inhibit inflammation and apoptosis, thus reduce the brain tissue damage[21,22].

The experiment detected the active extent of the lower extremity joints and the muscular tension, which showed that acupuncture treatment is effective. The active extent of the lower extremity joints was significantly increased and the size of the lower limb joints was significantly decreased. The results are consistent with the literature. The active extent of limb joints is related to the flexors of leg. The mechanism of acupuncture treatment to reduce muscular tension may be as follows .First: reduce local inflammation and reduce tissue damage, increases local blood flow of the brain damage area, and enhance neuronal damage compensatory function, promote the release of GABA, thereby decreased muscle tone; Second: inhibited the release of neurotransmitter acetylcholine from presynaptic membrane of neuromuscular junction ,directly reduce muscle tension and led to muscle relaxation[23-25].

In summary, the simulation of acupuncture treatment for spastic cerebral palsy rats showed that acupuncture can improve microcirculation and reduce inflammation, improvee the activity of rat models, which provide an experimental basis for the acupuncture of "Dazhui", "Baihui", "Renzhong" point to treat spastic cerebral palsy.

Conflict of interest statement

We declare that we have no conflict of interest.

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